Original Research Article

Detection of *Treponema denticola* by PCR in patients with different periodontal status

Varsha Chaudhary[1], Bhat Kishore[1], Sunil Rao[2], Manohar Kugaji[1] and Preeti Ingalagi[1]

[1]Department of Microbiology, Maratha Mandal's NGH   Institute of Dental
Sciences and Research Centre, Belgaum, India
[2]Department of Microbiology, Yenepoya Medical College, Yenepoya University,
Mangalore, India
Corresponding author

A B S T R A C T

Keywords

Aggressive
periodontitis;
Chronic
periodontitis;
PCR;
*Treponema
denticola.*

The aim of the study was to determine the prevalence of *T.denticola* by 16S rRNA-based PCR technique in subgingival plaque of periodontally diseased and healthy subjects and its relationship with the periodontal status. *T. denticola* is a highly motile, aerotolerent and anaerobic spirochete. It has been predominantly associated with the incidence and severity of human periodontal disease. As it is difficult to isolate and identify *T.denticola* from clinical plaque samples, currently, 16S rRNA-based PCR method continues to be one of the most sensitive , rapid, and cost-effective methods for determining the prevalence of such microorganisms. A total of three hundred subjects divided into three catogories namely; Chronic periodontitis (CP=100), Aggressive periodontitis (AP=100) and controls (C=100) were selected for the study in an age range of 20 to 50 years. Subgingival plaque samples were collected and forwarded for PCR analysis. The PCR was performed by using specific primers for the 16S rRNA gene of *T.denticola* and quantification was done using standard strain of *T.denticola*. Detection frequencies of *T.denticola* in plaque samples from AP patients and CP patients were reported much higher than those from healthy subjects. There was a significant difference between the PCR detections of CP and Control groups. Results of PCR depicted that Female were prone to chronic periodontitis than male in all age groups. This study demonstrated the utility of a 16S rRNA-based PCR detection method for identifying *T.denticola* and strong association between *T.denticola* and periodontitis.

Introduction

Periodontitis is a disease of multifactorial nature with pathogenesis related to several risk factors, including bacterial pathogens, host responses, and possibly certain genetic traits (Michalowicz *et al.,* 2000). It is characterized by the irreversible destruction of collegen fibers and other matrix canstituents of the gingiva, periodontal ligament, and alveolar bone around the teeth in conjunction with the formation of periodontal pockets due to the apical migration of the junctional

epithelium (Page and Kornman, 1997). The prevalence of periodontitis in humans is approximately 30%, of which 10-15 % is severe forms of the disease (Oliver *et al.,* 1998).

Spirochetes of genus Treponema are a major component of the bacterial flora of the oral cavity (Correla *et al*., 2004; Paster *et al.,* 2001). Of these, *Treponema denticola* has been predominantly associated with the incidence and severity of human periodontal disease such as chronic periodontitis, early-onset periodontitis and necrotizing ulcerative gingivitis (Loesche and Grossman, 2001). *T. denticola* is a highly motile, aerotolerent and anaerobic spirochete dwells in a complex and diverse microbial community and especially prevalent in the anaerobic gingival crevice (Paster et al., 2001). The accumulation of these bacteria and their products in the pocket may render the surface lining periodontal cells highly susceptible to lysis and damage (Sela, 2001).

In the periodontal pocket, *T. denticola* has been found close to the junctional epithelium. It has shown to adhere to epithelial cells, fibroblasts and to other bacteria as well as to matrix proteins (Thomas, 1996) and to collegen used as a barrier for guided bone generation (Sela *et al.,* 1999). *T.denticola* also shows nutritional interactions with other periodontal bacteria, especially *Porphyromonas gingivalis*. Moreover, due to oral treponemes having adherent properties to epithelial cells and coaggregation abitities to important biofilm-bridging organisms (Yao *et al*., 1996), the mechanical dispersal of dental plaque containing treponemes may lead to the colonization of new intraoral habits after periodontal therapy (Ehmke *et al*., 2004).

Methodologies involving culture, flourescence microscopy tests, DNA probes, and the polymerase chain reaction (PCR) are currently available for investigating periodontopathic bacterial infections in lesions. It is time-consuming work to evaluate the prevalence of these bacteria by the use of culture or fluorscence microscopy tests from the same clinical sample (Ashimoto et al., 1996; Takamatsu et al., 1999; Watanabe and Frommel, 1996). As it is difficult to isolate and identify *T.denticola* from clinical plaque samples, currently, 16S rRNA-based PCR method continues to be one of the most sensitive , rapid, and cost-effective methods for determining the prevalence of such microorganisms (Sakamoto *et al*., 1999; Sakamoto *et al*., 2001). The aim of the study was to determine the prevalence of *T.denticola* by 16S rRNA-based PCR technique in subgingival plaque of periodontally diseased and healthy subjects and its relationship with the periodontal status.

Materials and Methods

Sample Collection

The study was performed in the department of Microbiology, Maratha Mandal's NGH Institute of Dental Sciences,Belgaum. A total of three hundred subjects divided into three catogories namely; Chronic periodontitis (CP=100), Aggressive periodontitis (AP=100) and controls (C=100) were selected for the study. Informed consent was obtained from each participant before enrolling in the study. The study subjects were adults in an age range of 20 to 50 years and belonged to both the sex. The inclusion criteria for patients were: Chronic periodontitis, patients having periodontal pockets of $\geq$ 4mm, bleeding on probing, CAL of $\geq$ 3 mm^2 and radiograpfic

evidence of bone loss; Aggressive periodontitis, subjects with a pattern of severe destruction, with attachment loss of at least 4-7 or 8 mm, medical history nil and familiar aggregation of cases. Subject with a probing depth of 2-3mm and without any evidence of inflammation were selected as controls. Pregnant women and lactating mothers, those who had received systemic antibiotics and/or had undergone periodontal treatment during the last six months were excluded from the study.

Subgingival plaque samples were collected using 2-3 sterile paper points inserted for 30 sec. into the diseased sites of subjects with chronic and aggressive periodontitis. Same procedure has been followed for collection of samples from healthy ones. Paper points has to be pooled and placed in the eppendrof tubes containing transport media-Reduced Transport Fluid (RTF) and then samples were forwarded for PCR analysis.

PCR Analysis

DNA extraction was carried out for the samples. For this the samples were centrifuged at 5,000rpm for 5 min. Supernatant was discarded, 200 µl of fresh T.E. buffer was added and centrifuged for 3-4 minutes. Above procedure was repeated for 3-4 times with fresh T.E. buffer. Again the supernatant was discarded and 50 µl lysis buffer (Tris HCL: 50mM (pH 8.0) , KCL : 50mM ,MgCl2: 2.5mM, Tween 20: 0.45 %, Nodient P-40: 0.45%)and proteinase –K (10mg/ml) was added. It was kept in water bath for 2 hrs at 60^0C then kept in boiling water bath for 10 minutes. The extracted DNA is stored at -20^0C and used for PCR analysis.

The PCR was performed in a verity thermal cycler (Applied biosystem) by using specific primers for the 16S rRNA gene of *T.denticola*. PCR primers (Forward primer: 5'-TAA TAC CGA ATG TGC TTT ACA T-3' and Reverse primer: 5'- TCA AAG AAG CAT TCC CTC TTC TTC TTA-3') of 316 bp length were used in the current study. The PCR protocol was as follows: 95^0C for 5 min followed by 40 cycles of 95^0C for 30 S, 60^0C for 1 Min, 72^0C for 1Min, and a final step of 72^0C for 5 min. Amplified products were analyzed by 2% agarose gel electrophoresis, stained with 0.5 µgm/ml ethidium bromide, visualized and digitized using a BioRad Gel Doc 2000 system (Fig.1). As molecular size marker, 100bp DNA ladder (Chromous Biotech) was used. Quantification was done using standard strain of *T.denticola.* (Fig.1)

Results and Discussion

A Microbiological study was conducted to detect *T.denticola* by PCR in Chronic periodontitis (CP), Aggressive periodontitis (AP) and control groups (C). Comparison was done between the results obtained in all the above three groups. Detection frequencies of *T.denticola* in plaque samples from AP patients (67%) and CP patients (81%) were much higher than those from healthy subjects (55%, Graph 1). In Aggressive and Chronic periodontitis patients *T.denticola* was detected frequently at sites that showed deep periodontal pockets and severe attachment loss. There was a significant difference between the PCR detections of CP and Control groups (P value <0.001 is significant). While, there was no significant difference between the results of healthy (control group) and AP patients. Results were also compared against age & sex. Age wise distribution was done as 20-30, 31-40 and 41-50. Results of PCR

depicted that Female were prone to chronic periodontitis than male in all age groups. When compared to control group female in age group 41- 50 were most affected for chronic periodontitis i.e.the age of CP group was significantly greater than AP and healthy subjects. Age wise results of AP group were showing that female in age group 20-30 were more positive for *T. denticola* (Graph 2-4).

Bacterial infections may cause disease manifestations through multiple mechanisms, including, direct effects of bacterial products, effect of the host immune response to the organism, and the persistent actions of the host immune response after clearance of the organism (Lin *et al*., 2000). Yet it is controversial that the signs and/or symptoms of periodontal disease result from the presence of the microorganisms, the cause of periodontal breakdown in patients with AP and CP (Tamura *et al*., 2006). The present study was conducted to evaluate the prevalence of *T.denticola* in sub gingival plaque samples of patients with CP/AP and periodontally healthy subjects and its relationship with the periodontal status.
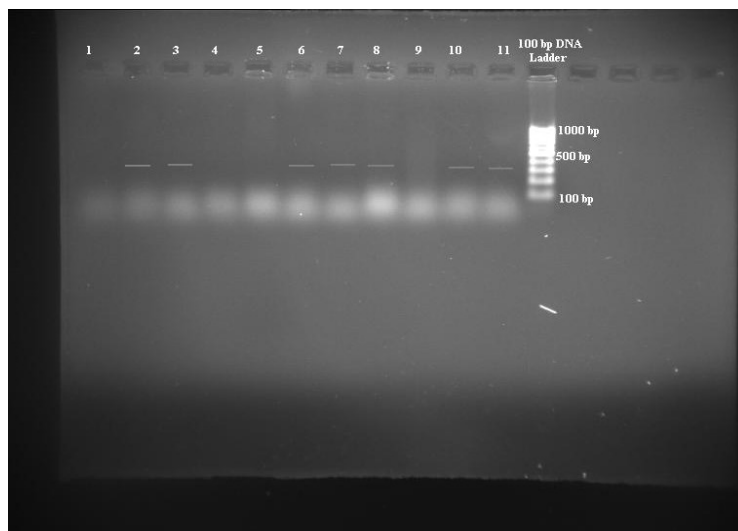
Several studies using subgingival plaque samples have shown that *T. denticola* is more frequently detected in periodontally diseased subjects than in healthy subjects (Riviere *et al*., 1995; Simonson *et al*., 1988; Takeuchi *et al*., 2003). The results of Statistics in the present study showed a positive association between CP subjects and *T.denticola*. This finding concurs with previous reports where *T.denticola* has been specifically implicated in CP[4,] (Sela, 2001). More recently, it has been also suggested that *T.denticola* may be one of the principal bacteria observed in AP

(Takeuchi *et al*., 2003). In this study, a significantly higher detection frequency of T.denticola was obtai ned in sub gingival plaque samples from CP patients (81%) in comparison with those from AP and healthy subjects (67% and 55% respectively). The differences in *T.denticola* detection between AP and healthy subjects were not statistically significant. These results suggested that the mechanism by which the severe and rapid periodontal breakdown occurs in AP might be independent of the presence of *T.denticola* and it is likely to be by the involvement of other bacteria with higher pathogenicity, such as *Tannerella forsythensis* and *P. gingivalis*, as noted by others (Takeuchi *et al*., 2003). Periodontal destruction in AP is also affected by several factors that affect the immune response of the host among them neutrophil function abnormalities (Page *et al.,* 1985) or execessive production of inflammatory cytokine/prostaglandin (Offenbacher, 1996).
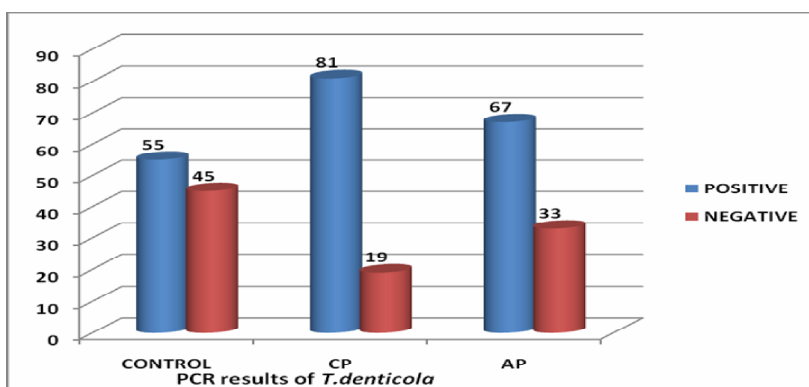
On the other hand, it is given that a pathogen may need to be in numbers sufficient to exceed a particular threshold to initiate disease, *T. denticola* may be found in healthy subjects who may be referred to as being in a carrier state (Watanabe and Frommel, 1996). Consequently, although the concentration of periodontal pathogens in sub gingival sites is the critical factor that will determine whether disease will occur or progress. The results of this study revealed that the patients in the CP group were significantly more positive for *T. denticola* than AP and healthy subjects.

In summary, the findings, when used in conjunction with an optimized clinical examination protocol, the assay may offer
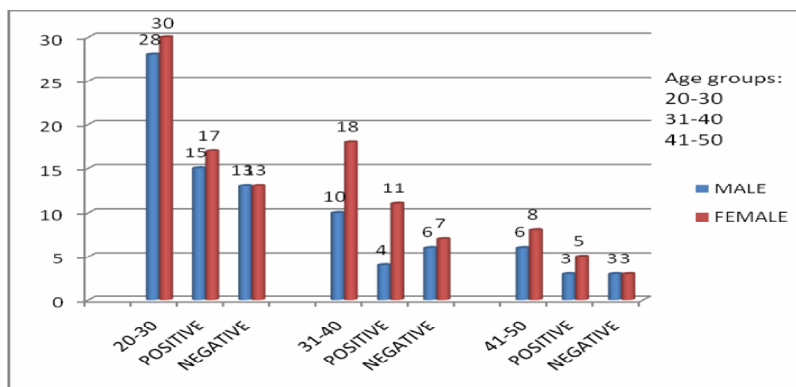
Fig.1 Lane numbers 2, 3, 6, 7, 8, 10 and 11 showing bands of amplified DNA products at 316 bp position in Chronic Periodontitis samples.
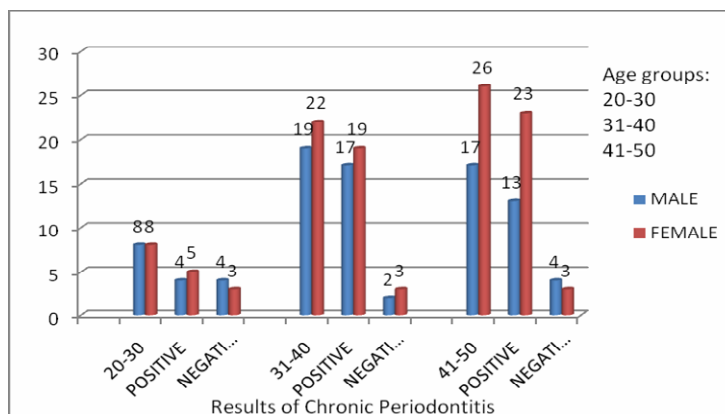

Graph.1 Showing PCR results for T.denticola.
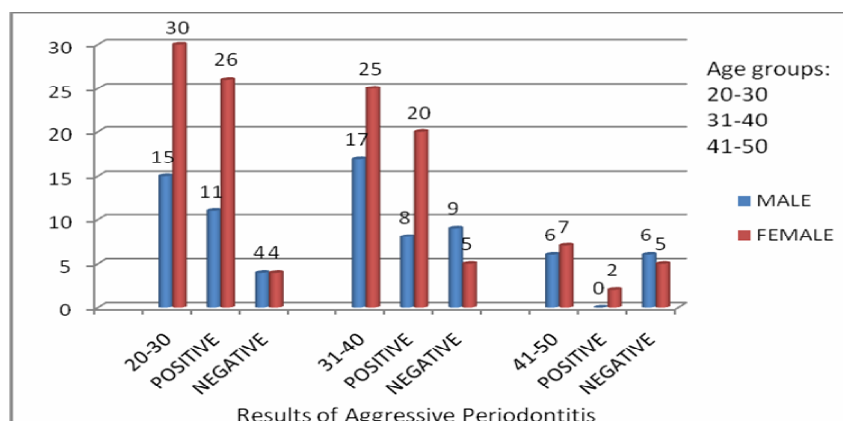

.

Graph.2 Showing Age wise results of control samples.

Graph.3 Showing Age wise results of Chronic periodontitis samples

Graph.4 Showing Age wise results of Aggressive periodontitis samples.

a rapid, useful, and cost-effective tool for monitoring the presence of *T.denticola* in sub gingival plaque samples from both healthy and diseased patients and correlating it with the amount and extent of periodontal breakdown.

The study concluded that 16S rRNA-based PCR technique is a rapid, useful, and cost-effective tool for monitoring the presence of *T.denticola* in sub gingival plaque samples from both healthy and diseased patients.

References

Ashimoto, A., et al. 1996. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. Oral. Microbiol. Immunol. 11: 266-273.

Correla, F.F., et al .2004. Genome size of human oral Treponema species by pulsed-field gel electrophoresis. Oral. Microbiol. Immunol. 19: 129-131.

Ehmke, B., et al. 2004. Intraoral dissemination of treponemes after periodontal therapy. Clin. Oral. Investig. 8:219-225.

Haffajee, A.D., et al. 1998. Subgingival microbiota in healthy, well maintained elder and periodontitis subjects. J. Clin. Periodontol. 25: 346-353.

Lin, B.O., et al., 2000. Soluble CD 14 levels in the serum, synovial fluid, and

cerebrospinal fluid of patients with various stages of Lyme disease. J. Infect. Dis. 181: 1185-1188.

Loesche, W.J., and Grossman, N.S. 2001. Periodontal disease as a specific albeit chronic, infection: diagnosis and treatment. Clin. MIcrobiol. Rev. 14: 727-752.

Michalowicz, B.S., et al. 2000. Evidence of a substantial genetic basis for risk of adult periodontitis. J. Periodontol. 71:1699-1707.

Offenbacher, S., 1996. Periodontal diseases: pathogenesis. Ann. Periodontol. 1: 821-878.

Oliver, .RC., L.J. Brown and Loe, H. 1998. Periodontal diseases in the United States population. J. Periodontol. 69: 269-278.

Page, R., and Kornman, K.S. 1997. The pathogenesis of periodontitis. Periodontol. 14: 9-11.

Page, R.C., et al. 1985. Clinical and laboratory studies of a family with a high prevalence of juvenile periodontitis. J. Periodontol. 56: 602-610.

Paster, B.J. et al., 2001. Bacterial diversity in human subgingival plaque. J. Bacteriol. 183: 3770-3783.

Riviere, G.R. et al., 1995. Subgingival distribution of *Treponema denticola, Treponema Socranskii*, and pathogen related oral spirochetes: prevalence and relationship to periodontal status of sampled sites. J. periodontal. 66: 829-837.

Sakamoto, M., et al .1999. Detection of *Treponema Socranskii* associated with human periodontitis by PCR. Microbiol. Immunol. 43: 485-490.

Sakamoto, M., et al. 2001. Rapid detection and quantification of five periodontopathic bacteria by real-time PCR. Microbiol. Immunol. 45: 39-44.

Sela, M.N., 2001. Role of *Treponema denticola* in periodontal diseases. Crit Rev Oral Bio. Med. 12: 399-413.

Sela, M.N., et al. 1999. Adherence of periodontopathic bacteria to bioabsorbable and non- absorbable barrier membranes in- vitro. Clin. Oral. Implants. Res. 10: 445-452.

Simonson, L.G., C.H. Goodman, J.J. Bial and Morton, H.E. 1988. Quantitative relationship of *Treponema denticola* to severity of periodontal disease. Infect. Immun. 56: 726-728.

Takamatsu, N., et al .1999. Effect of initial periodontal therapy on the frequency of detecting *Bacteoides forsythus, Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*. J .Periodontol. 70: 574-580.

Takeuchi, Y., et al. 2001. *Treponema Socranskii*, *Treponema denticola* and *Porphyromonas gingivalis* are associated with severity of periodontal tissue destruction. J. Periodontol. 72: 1354-1363.

Takeuchi, Y., et al .2003. Prevalence of periodontopathic bacteria in aggressive periodontitis patients in a japanese population. J. Periodontol .74: 1460-1469.

Tamura, K., et al .2006. Distribution of 10 periodontal bacteria in saliva samples from Japanese children and their mother. Arch. Ora.l Biol. 51: 371-377.

Thomas, D.D., 1996. Aspects of adherence of oral spirochetes. Crit. Rev. Oral. Biol. Med. 12: 399-413.

Umemoto, T., M. Li and Namikawa, I. 1997. Adherence of human oral spirochetes by collegen-binding proteins. Microbiol. Immunol. 41: 917-923.

Watanabe, K., and Frommel, T.O. 1996. *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans* and *Treponema denticola* detection in oral plaque samples using polymerase chain reaction. J. Clin. Periodontol. 23: 212-219.

Yao, E.S. et al. 1996. Interbacterial binding among strains of pathogenic and commensal oral bacterial species. Oral. Microbiol. Immunol. 11: 35-41.